

CLAIMS:

1. A preparative method for isolating RNA comprising an oligo- or polynucleotide from a sample, which method comprises:

- (a) treating the sample with a reactant capable of covalently modifying the 2'-OH position of the ribose rings of the RNA under conditions so that a proportion of the 2'-OH positions of the ribose rings bear a substituent; and
- (b) preparing isolated RNA therefrom by separating material containing the substituent from the sample on the basis of a property of the substituent.

2. A method according to claim 1, wherein step (a) is carried out in a reaction medium which comprises an organic solvent.

3. A method according to claim 2, wherein the organic solvent comprises an organic base.

4. A method according to claim 2 or claim 3, wherein the reactant comprises an acid anhydride, an acid chloride, a carboxylic acid or an N-acylimidazole.

5. A method according to claim 4, wherein the reaction medium further comprises an acylation catalyst.

6. A method according to any one of claims 2 to 5, wherein the reaction medium further comprises water.

7. A method according to any one of the preceding claims, wherein the RNA comprises mRNA, rRNA or viral RNA.

8. A method according to any one of the preceding claims, wherein the sample comprises a sample from a biological source.

9. A method according to any one of the preceding claims, wherein the sample includes DNA.

10. A method according to any one of the preceding claims, wherein the substituent comprises a solid phase.

11. A method according to claim 10, wherein the solid phase comprises benzoyl chloride polymer bound (BCPB) beads, silica particles or particles of a glass.

12. A method according to claim 10 or claim 11, wherein the solid phase is modified to introduce a reactive group which reactive group is capable of reacting with RNA to capture the RNA on the solid phase.

13. A method according to claim 12, wherein the reactive group is introduced by modifying the solid phase with a bi-functional acid halide.

14. A method according to any one of claims 1-9, wherein the substituent comprises a hydrophobic substituent.

15. A method according to claim 14, wherein the hydrophobic substituent comprises a substituent, OR,

wherein R comprises C₁-C₃₆ alkyl; C₁-C₃₆ alkenyl; C₁-C₃₆ alkynyl; C₁-C₃₆ haloalkyl; C₁-C₃₆ aminoalkyl; C₁-C₃₆ alkoxyalkyl; C₁-C₃₆ alkylthioalkyl; C₁-C₃₆ alkoxyalkoxyalkyl; C₁-C₃₆ haloalkoxyalkyl; C₁-C₃₆ aminoalkoxyalkyl; C₆-C₃₆ aryl; C₆-C₃₆ alkylaryl; C₆-C₃₆ arylalkyl; C₆-C₃₆ arylalkenyl; C₁-C₃₆ alkanoyl; C₁-C₃₆ alkenoyl; C₁-C₃₆ haloalkenoyl; C₁-C₃₆ haloalkanoyl; C₂-C₃₆ haloformylalkanoyl; C₁-C₃₆ C₁-C₃₆ aminoalkanoyl; C₁-C₃₆ azidoalkanoyl; C₁-C₃₆ carboxyalkanoyl; C₁-C₃₆ carboxyalkenoyl; C₁-C₃₆ carboxyalkynoyl; C₁-C₃₆ alkylaminoarylalkanoyl; C₁-C₃₆ alkoxycarbonyl; C₁-C₃₆ alkenyloxycarbonyl; C₁-C₃₆ alkylsulfonyl; C₆-C₃₆ arylalkanoyl; C₆-C₃₆ arylalkenoyl; C₆-C₃₆ aryloxyalkanoyl; C₆-C₃₆ alkylarylalkanoyl; C₆-C₃₆ haloarylalkanoyl; C₆-C₃₆ aminoarylalkanoyl; C₁-C₃₆ alkylsilanyl; C₁-C₃₆ trialkylsilanyl or C₁₂-C₂₈ diarylphosphano; or a substituent R', wherein R' comprises C₁-C₃₆ alkyl; C₁-C₃₆ alkenyl; C₁-C₃₆ alkynyl; C₁-C₃₆ haloalkyl; C₁-C₃₆ aminoalkyl; halo; amino; C₁-C₃₆ alkylamino; C₆-C₃₆ aryl; C₁-C₃₆ alkylaryl or C₁-C₃₆ arylalkyl.

16. A method according to claim 15, wherein the hydrophobic substituent comprises a C₄ to C₇ carbon chain or ring.

17. A method according to claim 16, wherein the reactant comprises butyric anhydride, pentanoic anhydride, hexanoic anhydride or benzoic anhydride.

18. A method according to claim 16 or claim 17, wherein the proportion of 2'-OH positions bearing the substituent is at least 10%.

19. A method according to claim 15, wherein the hydrophobic substituent comprises a C₈-C₁₂ carbon chain or ring.

20. A method according to claim 19, wherein the proportion of 2'-OH positions bearing the substituent is in the range 1 to 10%.

21. A method according to claim 15, wherein the hydrophobic substituent comprises a C₁₂-C₃₆ carbon chain or ring.

22. A method according to claim 21, wherein the proportion of 2'-OH positions bearing the substituent is up to 1%

23. A method according to any one of claims 14 to 22, wherein step (b) comprises contacting the treated sample from step (a) with a hydrophobic solid phase so as to bind the material containing the hydrophobic substituent and optionally washing the material bound to the solid phase.

24. A method according to claim 23, wherein the hydrophobic solid phase comprises hydrophobic particles.

25. A method according to claim 23 or claim 24, which further comprises a step of eluting the material bound to the hydrophobic solid phase by treating with a detergent, a chaotrope or a solvent, by lowering the salt concentration or by cleaving the substituent from the 2'-OH position of the ribose rings.

26. A method according to any one of claims 14 to 25, wherein step (b) comprises treating the treated sample from step (a) with a lyotropic salt to aggregate the material containing the hydrophobic substituent as an RNA precipitate, and isolating the precipitate.

27. A method according to claim 26, wherein the lyotropic salt comprises ammonium sulphate, an alkali metal chloride, magnesium chloride or calcium chloride.

28. A method according to any one of claims 14 to 22, wherein step (b) comprises treating the treated sample with a non-polar solvent to form a hydrophobic liquid phase which contains the material containing the hydrophobic substituent, and isolating the hydrophobic liquid phase.

29. A method according to claim 28, wherein the non-polar solvent comprises pentane, cyclohexane, toluene, benzene, light petroleum, xylene or hexane.

30. A kit for the preparative isolation of RNA comprising an oligo- or polynucleotide from a sample, which kit comprises:

(i) a reaction system for modifying the RNA to form a modified oligo- or poly-nucleotide in which a proportion of the 2'-OH positions of the ribose rings bear a substituent; and

(ii) a separation system for preparing isolated RNA by separating material containing the substituent from the sample on the basis of a property of the substituent.

31. A kit according to claim 30, wherein the reaction system comprises:

- (a) an organic solvent; and
- (b) a reactant capable of covalently modifying the 2'-OH position of the ribose rings of the RNA in the presence of the organic solvent.

32. A kit according to claim 31, wherein the organic solvent comprises an organic base.

33. A kit according to claim 31 or claim 32, wherein reactant comprises an acid anhydride, an acid chloride, a carboxylic acid or an N-acylimidazole.

34. A kit according to claim 33, which further comprises an acylation catalyst.

35. A kit according to any of claims 31 to 34, wherein the substituent comprises a solid phase.

36. A kit according to claim 35, wherein the solid phase comprises benzoyl chloride polymer bound (BCPB) beads, silica particles or particles of a glass.

37. A kit according to any one of claims 31 to 34, wherein the substituent comprises a hydrophobic substituent.

38. A kit according to claim 37, wherein the hydrophobic substituent comprises a substituent, OR, wherein R comprises C₁-C₃₆ alkyl; C₁-C₃₆ alkenyl; C₁-C₃₆ alkynyl; C₁-C₃₆

42. A kit according to claim 37, wherein the hydrophobic substituent comprises a C₈-C₁₂ carbon chain or ring.
43. A kit according to claim 42, wherein the proportion of 2'-OH positions bearing the substituent is in the range 1 to 10%.
44. A kit according to claim 37, wherein the hydrophobic substituent comprises a C₁₂-C₃₆ carbon chain or ring.
45. A kit according to claim 44, wherein the proportion of 2'-OH positions bearing the substituent is up to 1%.
46. A kit according to any one of claims 37 to 45, wherein the separation system comprises a hydrophobic solid phase for binding the material containing the substituent.
47. A kit according to claim 46, wherein the hydrophobic solid phase comprises hydrophobic particles.
48. A kit according to claim 46 or claim 47, wherein the separation system further comprises an elution medium for eluting RNA bound to the hydrophobic solid phase.
49. A kit according to any one of claims 37 to 45, wherein the separation system comprises a lyotropic salt for aggregating the material containing the hydrophobic substituent.
50. A kit according to any one of claims 37 to 45, wherein the separation system comprises a non-polar solvent for

haloalkyl; C₁-C₃₆ aminoalkyl; C₁-C₃₆ alkoxyalkyl; C₁-C₃₆ alkylthioalkyl; C₁-C₃₆ alkoxyalkoxyalkyl; C₁-C₃₆ haloalkoxyalkyl; C₁-C₃₆ aminoalkoxyalkyl; C₆-C₃₆ aryl; C₆-C₃₆ alkylaryl; C₆-C₃₆ arylalkyl; C₆-C₃₆ arylalkenyl; C₁-C₃₆ alkanoyl; C₁-C₃₆ alkenoyl; C₁-C₃₆ haloalkenoyl; C₁-C₃₆ haloalkanoyl; C₂-C₃₆ haloformylalkanoyl; C₁-C₃₆ C₁-C₃₆ aminoalkanoyl; C₁-C₃₆ azidoalkanoyl; C₁-C₃₆ carboxyalkanoyl; C₁-C₃₆ carboxyalkanoyl; C₁-C₃₆ carboxyalkynoyl; C₁-C₃₆ alkylaminoarylalkanoyl; C₁-C₃₆ alkoxycarbonyl; C₁-C₃₆ alkenyloxycarbonyl; C₁-C₃₆ alkylsulfonyl; C₆-C₃₆ arylalkanoyl; C₆-C₃₆ arylalkenoyl; C₆-C₃₆ aryloxyalkanoyl; C₆-C₃₆ alkylarylalkanoyl; C₆-C₃₆ haloarylalkanoyl; C₆-C₃₆ aminoarylalkanoyl; C₁-C₃₆ alkylsilanyl; C₁-C₃₆ trialkylsilanyl or C₁₂-C₂₈ diarylphosphano; or a substituent R', wherein R' comprises C₁-C₃₆ alkyl; C₁-C₃₆ alkenyl; C₁-C₃₆ alkynyl; C₁-C₃₆ haloalkyl; C₁-C₃₆ aminoalkyl; halo; amino; C₁-C₃₆ alkylamino; C₆-C₃₆ aryl; C₆-C₃₆ alkylaryl or C₁-C₃₆ arylalkyl.

39. A kit according to claim 38, wherein the hydrophobic substituent comprises a C₄ to C₇ carbon chain or ring.

40. A kit according to claim 39, wherein the reactant comprises butyric anhydride, pentanoic anhydride, hexanoic anhydride or benzoic anhydride.

41. A kit according to claim 39 or claim 40, wherein the proportion of 2'-OH positions bearing the substituent is at least 10%.

forming a hydrophobic liquid phase which contains the material containing the hydrophobic substituent.

51. A preparative device for isolating RNA comprising an oligo-or polynucleotide from a sample from a subject, which device comprises:

(i) a means for extracting the sample from the subject;

(ii) a reaction system for modifying RNA in the sample to form a modified oligo- or poly-nucleotide in which a proportion of the 2'-OH positions of the ribose rings bear a substituent; and

(iii) a separation system for preparing isolated RNA by separating material containing the substituent from the sample on the basis of a property of the substituent.

52. A device according to claim 51, wherein the means for extracting the sample from the subject comprises a syringe needle.

53. A device according to claim 51 or claim 52, wherein the substituent comprises a solid phase.

54. A device according to claim 53, wherein the solid phase comprises a membrane, a particle, a bead, a filter, a fibre, a gel, a strip, a matrix, a resin, a capillary or the walls of a vessel.

55. A device according to any of claims 51-54, wherein the sample comprises biological material.

- 68 -

56. A device according to claim 55, which device further comprises a filter for removing red and/or white blood cells.

ADD
A1

200003290001